*IN THE UNITED STATES PATENT AND TRADEMARK OFFICE*

Applicant: Kazuma TOMIZUKA *et al.*

Title: **METHOD FOR MODIFYING CHROMOSOMES**

Appl. No.: 09/763,362 Filing Date: 4/23/2001

Examiner: Thaian N. Ton Art Unite: 1632

**DECLARATION UNDER 37 C.F.R. §1.132**

Commissioner for Patents
Washington, D.C. 20231

Sir:

1. I, Kazuma Tomizuka, am an employee of Kirin Beer Kabushiki Kaisha ("Kirin"), located at 10-1, Shinkawa 2-chome, Chuo-ku, Tokyo, Japan. I have been employed by Kirin for the past 16 years. In my capacity as senior scientist/research head of chromosome engineering department in pharmaceutical research labs., I am responsible for working on the development and application of engineering technology for mammalian chromosome.

2. I have received a Ph. D. degree in life sciences from Tottori University in 2000. I have worked in the filed of molecular biology and genetics for 20 years. I have been a member of the Japanese association of molecular biology for 20 years and a member of the Japanese Gastroenterological association for two years.

3. I am a named co-inventor of U.S. Patent Application Serial No. 09/763,362, entitled "*Methods for Modifying Chromosomes*," which was filed on April 23, 2001 ("the application"). I do not have a personal interest in the outcome of the prosecution of this application.

4. The application includes claims directed to a recombinant chromosome that contains several co-joined human chromosome fragments, some of which contain loci for human antibody genes. The application discloses methods for making the recombinant chromosome.

5. In accordance with the technology claimed in the application, a recombinant chromosome acts as a vehicle that carries antibody gene clusters from different human chromosomes. Each gene cluster is surrounded by its natural chromosomal environment. Accordingly, the genes of any given cluster can be readily expressed without further intervention. That is, we do not need also to engineer expression cassettes or regulatory elements, such as promoters and terminators, into the chromosome to

ensure that each antibody gene is expressed. The recombinant chromosome also contains other elements, such as a centromere, to ensure it is replicated during cell division.

6. A recombinant chromosome is made up of human chromosome fragments that encode heavy and light antibody chains, respectively. Thus, antibodies are produced when those genes are expressed, which is a goal of our invention: to produce human antibodies in a non-human animal, such as a mouse.

7. I devised and conducted experiments involving the production of a recombinant chromosome as claimed. I included details and results of many of those experiments as Examples in the application.

8. Having read Examiner Thaian Ton's Office Action mailed October 5, 2005, I understand her to believe that some ambiguity affects the origin and creation of the human chromosome fragments that make up the recombinant chromosome.

9. In response to those concerns, and in response to her invitation that we do so (page 6 of the office action), I furnish this declaration to attest that the claimed chromosome (a) is made by reproducible methodology disclosed in the application and (b) does not require any particular or "essential" starting material.

10. I and my co-inventors devised a simple but sophisticated method for expressing human antibody genes in a non-human organism, such as a mouse. In essence, the inventive methodology requires three steps, as the application describes:

(a) isolating intact human chromosomes that contain clusters of antibody genes, such as chromosomes 2, 14, and 22, from a human cell;

(b) truncating the intact chromosomes with or without the use of irradiation, and isolating those fragments that contain antibody clusters; and

(c) ligating two of those resultant fragments with other chromosome fragments to produce the recombinant chromosome.

11. I understand that Examiner Ton questions whether (i) the step of truncating the intact chromosomes employs a method that fragments chromosomes randomly and (ii) the claimed recombinant chromosome actually is made up of one or more essential and unique chromosomal fragments.12. With

respect to point (ii), I understand the Examiner to assert that we appeared to use "a particular clone, which is an essential starting material" to produce the claimed fragments (page 7 of the office action). Specifically, I hear Examiner Ton to characterize our 6-1 clone as the "essential starting material" because it is a chromosome fragment that cannot be reproduced and, hence, is unique.13. With respect, I must say that this is a misunderstanding. Clone 6-1 is not a unique starting material for the purposes of making the recombinant chromosome.

14. With respect to point (i), three observations should help to clarify the origins of the genetic material that we used to produce the various recombinant chromosomes disclosed and claimed in our application:

(a) Clone 6-1 is essentially identical to A9/#22. Clone 6-1 is resistant to puromycin and G418 neomycin. See Example 26 at p. 149. The A9/#22 cell is the ancestral cell of 6-1 but is resistant to G418 neomycin only.

(b) A9/#22 of Example 2 is the same as "A9/#22neo" of Example 82. The "neo" designation emphasizes the presence of the G418 neomycin resistant gene on chromosome #22. While "A9/#22" lacks that specific designation, the A9/#22 clone also carries the G418 resistant gene. See Example 1 at p. 91, line 4 to p. 92, line 8.

(c) The G418 neomycin and puromycin resistance genes are used as selectable markers to identify cells that are cultured on neomycin- and puromycin-containing substrates.

15. We produced A9/#22-neomycin resistant cells by MMCT without irradiation according to the following steps: (1) we integrated a G418 resistant gene into chromosome #22 of human normal fibroblast cells (HFL-1); (2) we then fused those cells with mouse A9 cells via MMCT and cultured the fused cells on G418 selective media; and finally (3) we identified G418 resistant cells.

16. We screened those resistant cells using PCR and fluorescence *in situ* hybridization (FISH) and identified which cells contained an unfragmented human chromosome #22. We called these cells "A9/#22." Please see pp. 99-102 of the application.

17. Our Example 2 describes this sequence of events and details treatment of non-irradiated cells and irradiated cells. Accordingly, we report in Example 2 that clones "E14/#22-9" and "E14/#22-10" were created by fusing "nonirradiated" microcells with ES cells. We found that each of these clones retained an essentially intact chromosome #22. Please see p. 101, line 27 to p. 102 of the application.

18. Example 82 describes how we transferred chromosome #22 of A9/#22neo cells into chicken DT40 cells, and how we found that clone "52-18" contained an intact human chromosome #22. Please see p. 274, lines 2-4 of the application.

19. Thus, it is not the case that our examples, especially Example 2, describe clones that are "generated by irradiation of the A9/#22 microcells" (p. 5 of the office action). In fact, the G418-resistant chromosomes of the A9/#22 and A9/#22neo cells, described in Examples 2 and 82, are neither fragmented nor the result of irradiation.

20. We took that unfragmented chromosomal #22 "starting material" and cleaved it at its LIF gene locus *specifically*, via telomere truncation. We ligated the desired fragment to a chromosome #14 fragment to produce the human artificial chromosome, "λHAC." Please see Examples 83, 93, and 97.

21. Applying this simple but sophisticated method, as described elsewhere in the application, we also constructed the human artificial chromosome "κHAC." That is, we ligated specific fragments that we created in the same way, from intact copies of chromosomes #2 and #14.

22. Overall, therefore, we were able: (a) routinely to obtain mouse A9 cells that contained intact copies of human chromosome #2, #4, #14, and #22; and (b) specifically to truncate those intact chromosomes at any desirable locus, generating fragments that we could then ligate to produce a recombinant chromosome, as claimed in the application.

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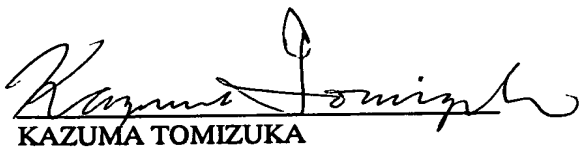
I hereby declare that all the statements made herein of my known knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Feb. 1, 2006
Date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